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(71) Applicant: MIRAGEN, INC. [US/US]; Suite 160, 6 Morgan, Irvine, CA 92718 (US).

(72) Inventor: UNGER, Thomas, F.; 23 Avignon, Newport Coast, CA 92657 (US).

(74) Agents: WITT, Evan, R. et al.; Madson & Metcalf, Suite 950, 170 South Main Street, Salt Lake City, UT 84101 (US).

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(54) Title: ANTIBODY PROFILE LINKED DIAGNOSTIC TESTING

(57) Abstract

The present invention is a method for discriminating and positively identifying the source of a biological sample used for diagnostic testing by linking diagnostic test results to an antibody profile of the biological sample. The invention is based on the principle that humans and other animals have unique sets of antibodies referred to as individual-specific antibodies or IS antibodies. When IS antibodies are reacted with a random number of antigens, such as human HeLa cell antigens, certain IS antibodies specifically bind to certain antigens forming immune complexes. When IS antibodies from different individuals are reacted with the same set of antigens, different immune complexes form. The highly unique combination of immune complexes that form generates a biological signature which is referred to as an antibody profile (AbP) or antibody fingerprint. By generating an AbP of each biological sample used for diagnostic testing, the present invention simultaneously positively identifies the origin of the biological sample assayed.

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# ANTIBODY PROFILE LINKED DIAGNOSTIC TESTING

## 1. The Field of the Invention

The present invention is directed to a novel method for discriminating and positively identifying the source of a biological sample used for diagnostic purposes. More specifically, the present invention links diagnostic test results to an antibody profile of the biological sample, and thus simultaneously positively identifies the source of the biological sample used for diagnostic testing.

## 2. Technical Background

It is often important to determine with a high degree of accuracy the identity of an individual. For example, in law enforcement, it is often critical to the investigation to determine the source of biological samples left at the scene of a crime. Accurate identification of individuals is also important in determining paternity and maternity. This is particularly important in a hospital setting where the identity of newborns is not readily apparent from physical characteristics such as fingerprinting. Identification of individuals is also important in time of war to ascertain the identity of soldiers or even to appropriately match body parts that have been severed.

In another context, it is important that results from biological tests be linked to the appropriate individual. In drug testing, for example, there is great incentive on the part of individuals consuming illegal drugs to substitute a urine or blood sample from an individual that does not consume drugs in order to avoid the legal consequences of testing positive. Similar incentives exist for athletes and animals used in competition. Therefore, it would be useful to determine whether the biological sample tested for illegal drugs indeed corresponds to the individual or animal in question.

A related, but far more serious concern, is the proper identification of blood samples and other biological samples. Due to the prevalence of AIDS, hepatitis B, cytomegalovirus, and other blood borne pathogens in the blood supply, the need for laboratory quality assurance is critical. Currently, there are numerous methods for detecting pathogens in the blood supply, including assays that detect pathogen specific antibodies, antigens, enzymes, DNA and even combinations of these techniques. Many of these assays are highly sensitive and specific for the given pathogens.

Nevertheless, the potential for false-positives and false-negatives exists. For example, there is a window between the time an individual becomes infected with a pathogen and the

development of a detectable immune response. Thus, an individual who is in fact positive may test negative for the pathogen because the level of the pathogen is so low that pathogen specific antibodies, antigens, enzymes and DNA are not detectable when the test is administered. The pathogen would be detectible a few weeks later, once the pathogen population has expanded and the individual's immune system has mounted an immune response against the pathogen.

Consequently, a two-tiered testing strategy is usually employed to ensure the accuracy of the initial test. For example, a blood sample may be tested using a highly sensitive enzyme or radio-immunoassay followed by a more specific method such as an immunoblot, immunofluorescent or immunoprecipitation assay. Not surprisingly, a small percentage of individuals who tested negative for the pathogen the first time subsequently test positively. These persons are considered to be positive for the pathogen and it is assumed that the first test was taken during the window when the pathogen levels and immune response to the pathogen were too low to detect. Conversely, some persons who first tested positive for the pathogen subsequently test negatively. These persons are referred to as seroreverters. In these instances, the initial positive result has been considered to be a false positive due to splash or other contamination and the individual is considered not to harbor the pathogen.

In one study, Ascher, D. P. and C. Roberts, *J Acquired Immune Defic Syn* 6: 241-244 (1993), human immunodeficiency virus (HIV) test results of the U.S. Department of Defense were analyzed to determine what percent of seroreverters were actually due to different or disconcordant patient samples. In other words, how many individuals were labeled seroreverters because the first and second biological samples tested in fact belonged to different individuals and thus their HIV test results were improperly compared.

Of the 1,402 persons that tested positive for HIV, 15 (1%) tested negatively on the subsequent test and thus labeled seroreverters. Of the 15 seroreverters, 7 (42%) were shown to be from comparing biological samples and results from different individuals. Therefore, 7 persons considered by the U.S. Department of Defense to be HIV negative, were potentially HIV positive. These results are disturbing considering the potential consequences to the individual and society of such misidentification errors.

Other studies confirm that misidentification errors are common in the industry. Studies by Roy et al., *JAMA*, **269**: 2876-2879 (1993); Sheppards et al., *J Acquired Immune Defic Syn*

4: 277-283 (1991); Imagawa et al., *N Eng J Med*, **320**: 1458-1462 (1989); and Taswell et al., *Arch Pathol Lab Med*, **118**: 405-410 (1994), all report errors in sample identification of between 1.3 and 8.1%. Indeed, consistent with Ascher and Roberts' study, a Transfusion Safety Study funded by the National Institute of Health reported that 60% of laboratories errors are due to sample mislabeling and mishandling. Other studies confirm that in addition to mislabeling and mishandling errors, reporting and recording errors account for a significant amount of sample identification errors.

Traditionally, several methods have been used to aid in identifying individuals and biological samples, including blood typing, fingerprinting, photographs and serological assays based on Human Leukocyte Antigen gene loci (HLA complex). These methods while useful to a certain degree, have several shortcomings described in US Patent No. 5,270,167 to Francoeur which is hereby incorporated by reference.

Recently, DNA based identification methods have been used to identify or discriminate between individuals and biological samples. DNA based identification methods require the presence of cellular material or other source of DNA. Not all biological samples, however, contain a source of DNA. For example, serum or plasma is free of cells and therefore DNA based identification methods cannot be used to correct serum or plasma misidentification errors due to mishandling and mislabeling.

Moreover, in theory, every individual's DNA make-up is different, and thus DNA-based testing is highly discriminatory. Currently, however, it is impossible to test an individual's entire DNA make-up. Thus, techniques such as restriction fragment length polymorphism (RFLPs) and polymerase chain reaction (PCR) compare only seven points on the DNA which are thought to be highly variable between individuals. Because of the limited DNA regions tested, many scientists argue that DNA-based assays are useful only at discriminating between individuals, not as a positive identification method.

Even as a discrimination method, DNA-based identification has its limitations. For example, using DNA-based identification techniques, it is difficult to discriminate between genetically identical individuals, such as twins or even close relatives. Furthermore, PCR-based assays are highly susceptible to contamination. Results, therefore, are readily compromised and unreliable.

Apart from the technical difficulties, the economic realities of DNA-based testing make its widespread use difficult. It costs nearly half a million dollars to equip a DNA lab and technician training generally takes between 4 and 6 weeks. Based in part on these set-up costs, RFLPs and PCR identification typically cost $1,000 and $250 dollars per assay, respectively. For most institutions, the cost of DNA-based testing is prohibitively high for use as a routine identification method.

Results from DNA-based testing are also relatively slow. RFLP and PCR testing generally takes between 8 weeks and 2-3 days, respectively. A delay of several days is impractical or impermissible in many instances. For example, it will be appreciated that patients waiting to receive organ transplant or blood transfusion often cannot wait 2 or 3 days for the results of DNA-based assays.

From the foregoing, it will be appreciated that it would be an advancement in the art to provide a method that could both highly discriminate between and positively identify individuals. It also would be an advancement if the method was easy to administer, required small biological samples, and was cost effective.

In addition, it would be an advancement if the identification method were linked to diagnostic testing thus providing assurance that the biological sample tested in fact belonged to the individual in question. It would be a further advancement in the art if the method could be packaged in the form of a kit and required little specialized training to practice. It would be yet another advancement if the method were automated and the automated equipment were inexpensive so that accurate identification results could be obtained quickly without having to send the biological sample to an independent lab for testing. Finally, it would be an advancement if the identification data could easily be digitized and stored in a computer for easy access and reference.

Such a system is disclosed and claimed herein.

## 3. BRIEF SUMMARY OF THE INVENTION

The present invention is directed to a novel method for discriminating and positively identifying the source of a biological sample used for diagnostic purposes by linking the diagnostic test results to an antibody profile of the biological sample. The invention is based on the principle that humans and other animals have unique sets of antibodies. These

antibodies are referred to as individual-specific antibodies or IS antibodies. When IS antibodies are reacted with a random number of antigens, such as human HeLa cell antigens, certain IS antibodies specifically bind to certain antigens. IS antibody/antigen binding complexes are generally referred to as immune complexes. The number of immune complexes that form depends on the number of antigens and the concentration of IS antibodies used. As many as $10^{23}$ different immune complexes may form. When IS antibodies from different individuals are reacted with the same set of antigens, different immune complexes form. The highly unique combination of immune complexes that form, or fail to form, generates a biological signature which is referred to as an antibody profile (AbP) or antibody fingerprint.

The present invention links AbP with diagnostic testing such that the AbP identifies the source of the biological sample used to generate the diagnostic results. Moreover, AbP's from subsequent diagnostic testing can be compared to ensure that the biological sample used was obtained from the same source.

In one embodiment, for example, an antibody profile can be linked to an HIV diagnostic assay. The assay is performed in a reaction vessel with a plurality of wells or reaction cells. A first subset of reaction cells is used to generate an AbP and a second subset of reaction cells used for the purposes of diagnosing HIV. The results obtained from the second subset of reaction cells is generally referred to as a diagnostic profile. With the exception of reaction cells used for negative and positive controls, in one embodiment the first subset of reaction cells are bound with antigens derived from HeLa cells and the second subset of reaction cells are bound with antigens that are specific for antibodies directed at HIV.

To each reaction cell, biological sample diluted in the appropriate buffer is added. The antigen/biological sample mixture is allowed to react for a time sufficient to permit immune complexes to form between the bound antigens and individual-specific antibodies in the biological sample, including any antibodies specific to HIV. Each reaction well is then washed to remove nonspecific binding and the immune complexes are identified. In one embodiment, the immune complexes are identified using a detector molecule such as an anti-immunoglobulin antibody or secondary antibody that has been labeled with an enzyme, a fluorophore, or a chemiluminescent substance. The secondary antibody binds to immune complexes, but not antigen alone. After a second washing, the amount of secondary antibody

in each reaction cell is quantified. For example, if the secondary antibody was labeled with an enzyme, the appropriate enzyme substrate would be added and the amount of product, which is usually colored, determined spectrometrically. Finally, the spectrometric data from the AbP and the diagnostic profile would be digitized and stored in a computer.

Therefore, the digitized AbP linked diagnostic test results can be compared to previous or subsequent AbPs to ensure that the biological sample tested was from the same source or the source in question. If the AbPs do not match, the biological samples were mislabeled or derived from a different source. Apart from discriminating between sources of biological samples, the AbP can then be used to positively identify the source of the biological sample. This could be done by searching an AbP databank until an appropriate match is found.

The AbP linked diagnostic profile of the present invention, therefore, offers several advantages over current diagnostic methods in the art which rely on manual identification methods or cumbersome physical identification methods to determine the source of a biological sample. First, the present method provides absolute concordance between biological samples and diagnostic testing. Second, the method of the present invention is easy to administer, reliable, stable and cost effective. Third, the necessary reagents and reaction vessels required to practice the present method can be packaged in a kit and performed by personnel with little specialized training. Fourth, the method of the present invention can be automated and the necessary automating equipment is relatively inexpensive so that accurate identification results can be obtained quickly in-house without requiring the biological sample to be sent to an independent lab for testing. Finally, AbP data can be easily digitized and stored in a computer for easy access and reference.

These and other objects and advantages of the present invention will become apparent upon reference to the accompanying drawings and graphs and upon reading the following detailed description and appended claims.

## 4. SUMMARY OF THE DRAWINGS

A more particular description of the invention briefly described above will be rendered by reference to the appended drawings and graphs. These drawings and graphs only provide information concerning typical embodiments of the invention and are not therefore to be considered limiting of its scope.

**Figure 1** is a perspective view of a typical reaction vessel of the present invention. Figures 1a and 1b are expanded views of two reaction cells illustrating a different reactant bound to each reaction cell.

**Figure 2** illustrates an automated process for practicing the method of the present invention.

**Figure 3** is a perspective view of a typical reaction vessel of the present invention after a biological sample and a secondary antibody have been allowed to react with the reactant in each reaction cell. Figure 3a is an expanded illustration of a reaction cell where an IS antibody is not bound to the reactant, and hence no fluorescent-labeled secondary antibody is bound to the IS antibody and no signal is detected. Figure 3b is an expanded illustration of a reaction cell where an IS antibody is bound to the reactant, and hence fluorescent-labeled secondary antibody is bound to the IS antibody and a signal is detected.

**Figure 4** illustrates fluorescent light emissions from reaction cells were fluorescent-labeled secondary antibody is bound to IS antibody and a detector for measuring fluorescent light emissions.

**Figure 5** illustrates a digitized AbP and diagnostic profile where each number corresponds to a reaction cell of the reaction vessel.

## 5. DETAILED DESCRIPTION OF THE INVENTION

The present invention is directed to a novel method for discriminating and positively identifying the source of a biological sample used for diagnostic purposes. More specifically, the present invention provides a method for assaying analytes in a biological sample and simultaneously identifying the origin of the biological sample assayed.

A more particular description of the invention briefly discussed above will be rendered by reference to the appended drawings. The drawings and accompanying description provide information concerning a typical embodiment of the invention and therefore is not to be considered limiting of its scope. With reference to Figure 1, the method of the present invention is carried out in a reaction vessel **10**. Reaction vessel **10** has a plurality of cylindrical shaped reaction cells **12**. Reaction cells **12** are divided into two subsets: a first subset of reaction cells **14** for the purpose of obtaining an antibody profile and a second subset of reaction cells **16** for the purpose of obtaining a diagnostic profile.

As illustrated in Figure 1a and 1b, except for reaction cells used as negative and positive controls, different reactants **18** are bound to reaction cells **12**. In one preferred embodiment, reactants **18** are antigens. In the first subset of reaction cells **14**, reactants **18** can be random antigens isolated from HeLa cells or known recombinant antigens. In the second subset of reaction cells **16**, reactants **18** are known antigens specific for antibodies corresponding to the diagnostic testing to be performed. For example, if the purpose of testing is to diagnose whether an individual has HIV-1 or hepatitis B, reactants **18** in the second set of reaction cells **16** would be antigens directed at HIV-1 or hepatitis B, respectively. In addition, as a positive control, a second reactant (not shown) could be included in each well as a positive control. For example, a reactant that binds human immunoglobulins may be included as a positive control that a sample was applied and that the sample is of human origin. The signal generated by the positive control would, of course, be distinct from the signal generated by the biological sample. For example, the positive control could emit light at a different wavelength. Once reactants **18** are bound, reaction vessel **10** containing reactants **18** can be stored under refrigeration and used at a later time.

As illustrated in Figure 2, the process for generating an AbP and diagnostic profile may be automated. Reaction vessel **10** and biological sample **31** are loaded on a conveyer **34** where the following steps are performed:

(1) The personal information on a biological sample **31** to be tested is either read by a scanner **30** or manually entered into a computer **32**. As will be discussed below, the personal information is automatically linked with the AbP that will be generated from biological sample **31**.

(2) An equal amount of biological sample **31** is contacted with reaction cells **12**. With reference to Figures 2 and 3, individual specific antibodies **20** in biological sample **31** are allowed to form immune complexes with reactants **18** bound to reaction cells **12**.

(3) After an appropriate incubation period, excess biological sample **31** is removed and reaction cells **12** are washed with a suitable buffer such as phosphate buffered saline.

(4) In one preferred embodiment, the immune complexes are identified using a secondary antibody. For example, immune complexes between reactants **18** and IS antibodies **20** are identified using a fluorescent-labeled anti-immunoglobulin **22**, such as fluorescein-labeled anti-IgG. Anti-immunoglobulin **22** recognizes IS antibodies **20**, but not antigen **18**.

(5) After an appropriate incubation period, excess fluorescent-labeled anti-immunoglobulin **22** is removed and reaction cells **12** are washed with a suitable buffer.

(6) Immune complexes are detected. As illustrated in Figure 3, it will be appreciated that depending on the repertoire of IS antibodies **20** in biological sample **31**, in some reaction cells **12** immune complexes will form and in others no immune complexes will form. As best illustrated by Figure 3b, in reaction cells **12** where immune complexes formed, fluorescent-labeled anti-immunoglobulin **22** is bound to IS antibodies **20**; and as best illustrated by Figure 3a, in reaction cells **12** where immune complexes did not form, there is no fluorescent-labeled anti-immunoglobulin **22** bound to IS antibodies **20**. With reference to Figure 4, those reaction cells **12** where immune complexes formed, and hence contain fluorescent-labeled anti-immunoglobulin **22**, will emit fluorescent light **23**. In the first subset of cells **14**, the pattern of fluorescent light **23** corresponds to the individual's unique AbP. In the second subset of cells **16**, the pattern of fluorescent light **23** corresponds to the individual's diagnostic profile. As such, the individual's diagnostic profile is linked to the individual's unique AbP. With continued reference to Figures 4, detector **24** scans reaction cells **12** and at every position measures whether fluorescent light **23** is being emitted and the strength of the emission.

(7) The pattern of fluorescent light **23** is digitized. The pattern is digitized by ascribing a numeral "0" to reaction cells **12** that do not emit fluorescent light **23** and the numeral "1" to reaction cells **12** that do emit fluorescent light **23**. As illustrated in Figure 5, once reaction cells **12** have been ascribed a numerical value, the AbP will be stored in computer **32** as a digitized AbP **26** with each numeral corresponding to a single reaction cell **12** of reaction vessel **10**.

Once the process is complete, the individual's personal information, AbP and diagnostic profile are all linked in computer **32**. Thus, by comparing the individual's digitized AbP **26** with a previously digitized AbP, the source of biological sample **31** can be identified. If the digitized AbP **26** of the individual does not match a previous AbP, it is assumed that biological sample **31** was mislabeled and a second biological sample can be tested. In addition, a digitized AbP database **28** containing the AbP of numerous individuals can be searched using digitized AbP **26** in order to positively identify the source of the biological sample.

To better understand the details of the invention, the discussion below is divided into five sections corresponding to the five basic steps of the present invention which include: (1)

obtaining suitable reactants; (2) obtaining a suitable reaction vessel; (3) obtaining a biological sample and reacting the biological sample with the reactants; (4) detecting and quantifying reactions, or lack of reactions, between reactants and analytes in the biological sample; and (5) digitizing the results.

5

### 5.1 Reactants

The first step in practicing the method of the present invention is to obtain reactants to generate an AbP and a diagnostic profile. A reactant is defined broadly to include any molecule which can be used to measure the presence of a molecule in a biological sample referred to as an analyte. Hence reactants can be bacterial, viral, or mammalian cell antigens, hormones, drugs, receptors, tumor markers, or numerous other substances. Moreover, the reactant can be natural or synthetic, a nucleic acid or a peptide, or combinations thereof.

For purposes of generating an AbP, the reactant is an antigen. An antigen is defined as a molecule that is bound by an antibody. In one embodiment, antigens are isolated from HeLa cells as described in Francoeur et al., *J Immunology*, **136**:1648 (1986) which is incorporated by reference. Briefly, HeLa cells are grown in standard medium under standard tissue culture conditions. Confluent HeLa reaction cells are then rinsed with phosphate buffered saline (PBS), lysed with detergent and centrifuged to remove insoluble reaction cellular debris. The supernatant contains approximately 10,000 immunologically distinct antigens suitable for generating an AbP.

There is no requirement that the antigens used to generate an AbP be known. All that is required is that the source of the antigens be consistent such that a reproducible AbP profile can be generated at a later time. For example, the HeLa cell supernatant containing the antigens can be separated on a size exclusion column. Each fraction collected could represent a unique set of antigens for purposes of generating an AbP. Thus, even though the antigens are unknown, reproducible AbP can be generated if the HeLa cell antigens are isolated and separated using the same method. Other methods such as phage-display technology well know in the art may be used to reproducibly generate antigens. See Cannon et al., "Phage-Display Technology," *IVD Tech.* **22** (1996).

Alternatively, reactants can be synthesized using recombinant technology well known in the art. Genes that code for many viral and bacterial proteins have been cloned and thus

large quantities of highly pure proteins can be synthesized quickly and inexpensively. Likewise, the genes that code for many eukaryotic and mammalian membrane bound receptor, growth factors, cell adhesion molecules, steroids and regulatory proteins have been cloned and are useful as reactants. Many recombinant proteins such as transforming growth factor α, acid and basic fibroblast growth factor, interferon, insulin-like growth factor, and various interleukins from different species are commercially available from, for example, Promega Corporation, Madison, Wisconsin.

In most instances, the entire polypeptide need not be used as a reactant. For example, any size or portion of the polypeptide that contains at least one epitope (i.e., the critical portion of the polypeptide recognized during an immune response) will suffice for generating an AbP. In another example, the reactant may be the catalytic region or subunit of a protein which catalyzes a reaction using an analyte in the biological sample, such as the catalytic region or subunit of a protein kinase.

One skilled in the art will appreciate that the signal-to-noise ratio of the AbP and diagnostic profile will improve with the purity of the reactants used. Hence, depending on the signal-to-noise ratio desired, the reactant can be further purified, for example, by ammonium sulfate precipitation, size exclusion, dialysis or other methods well known in the art.

### 5.2 Reaction Vessel

Once reactants have been isolated or synthesized, they are added to a reaction vessel. The exact configuration of the reaction vessel is not critical to the invention. The reaction vessel need only be able to hold a sufficient number of reactants to generate an AbP and a diagnostic profile. Regardless of the material or configuration of the reaction vessel, each location where at least one reactant is contained or bound, or could be contained or bound, is referred to as a reaction cell. The reaction vessel could be a multiwell gel electrophoresis apparatus or a synthetic membrane capable of binding reactants. In one embodiment, the reaction vessel is a microcassette configuration such as Corning™ disposable sterile polystyrene 96-well plates commercially available from Sigma Chemical® Company, St. Louis, MO, MicroAmp® disposable multiwell plates commercially available from Roche Molecular Systems, Branchburg, NJ, or similar multiwell plate well known in the art. Multiwell plates with lids are preferred because they prevent cross contamination. Moreover, the reaction

vessel may be constructed from a number of different materials, including polystyrene, glass, cellulose membrane, metal or silicone.

With the exception of the reaction cells used as negative controls, at least one reactant is added to a plurality of reaction cells. Reactants may be in solution, bound to the reaction vessel, or bound to another surface, such as latex, polystyrene, magnetic, or glass beads, which are in turn confined or bound to the reaction vessel. In one preferred embodiment, the reactants are bound to a solid phase such as the reaction vessel. Many of compounds well known in the art naturally adsorb reactants, such as proteins. For example, polystyrene is capable of physically adsorbing proteins which remain attached to the solid phase even during subsequent manipulations. It is also well known in the art that compounds that do not adsorb reactants may be coated with substances which permit reactants to remain securely bound to the solid phase.

Alternatively, the reaction vessel could be a chip on which the reactants would be directly synthesized. Polypeptide and nucleic molecules can be synthesized directly in the reaction cells. See C&EN, June 6, 1994; *Proceedings of the National Academy of Science USA*, **91**: 5022 (1994) and references cited therein which are incorporated by reference.

In addition to providing a reaction medium, the reaction vessel may directly or indirectly participate in identifying a reaction between the reactant and the biological sample. For example, the solid support may be an electrode or crystal. Thus, the properties of the reaction vessel would change when, for example, an antibody binds an antigen. The solid support may also be utilized to initiate the catalysis of any of a number of physical or chemical reactions for the purposes of detecting whether a certain analyte is present in the biological sample. Finally, the reaction vessel may be magnetic or may become magnetic to aid in separation processes which utilize magnetic beads.

### 5.3 **Biological Sample**

The source of the biological sample can be from any animal which generates individual specific (IS) antibodies. Currently, humans, dogs, cats, mice, horses, cows and rabbits have all shown to possess IS antibodies. Moreover, determining whether a particular animal generates IS antibodies can be easily determined by one skilled in the art using common immunological techniques, including those taught in U.S. Patent No. 5,270,167 to Francoeur.

The biological sample can be from various bodily fluids and solids, including blood, saliva, semen, serum, plasma, urine, amniotic, pleural or cerebrospinal fluid. Depending on the detection method used, it may be required to manipulate the biological sample to attain optimal reaction conditions. For example, the ion concentration of the biological sample may be adjusted for optimal immune complex formation, enzymatic catalysis, DNA hybridization, or DNA synthesis. It is also contemplated that the portion of the biological sample used to generate an AbP can be treated differently than the portion of the biological sample used to generate the diagnostic profile. For example, a portion of the biological sample could be adjusted for optimal immune complex formation and another portion of the sample could be adjusted for optimal PCR condition.

Once the biological sample is optimized for reaction conditions, a small portion of the sample is added to each reaction cell of the vessel. The amount of biological sample required per reaction cell will depend on the biological assay performed and the sensitivity of the detection method used. Generally, just a few microliters of biological sample is sufficient to generate an AbP.

Analytes in the biological sample will interact with the reactants in the reaction vessel. The reactions between the analyte and the reactants will generate (1) an AbP and (2) a diagnostic profile. A detailed description of how these two profiles are generated is described below.

### 5.3.1 *Antibody Profile (AbP)*

An AbP is generated by multiple immune complex formations between reactants and IS antibodies in the biological sample. The immune system is a complex network of cells with the ability to recognize and rid the body of pathogens, as well as non-pathogenic foreign material. One way the immune system rids the body of pathogens and foreign substances is to generate large quantities of unique antibodies. Antibodies are molecules that are able to bind to molecules on the surface of the pathogen or foreign substances. The molecules to which antibodies bind are referred to as antigens. As defined above, the binding of an antibody to an antigen is referred to as an immune complex.

An animal can generate millions of different antibody molecules. This capacity and underlying antibody repertoire has evolved to ensure that the immune system can generate an

antibody that can form an immune complex with an unlimited number of antigens. Moreover, even though individuals may have antibodies towards the same antigens, each antibody will usually recognize a different region (epitope) on the antigen and, if appropriately assayed, these antibodies are therefore unique to that individual.

In addition, binding of antibodies to the pathogen activates reaction cells in the immune system which engulf and destroy the pathogen. Once the pathogen is defeated, the levels of antibodies directed at the pathogen decrease. However, cells in the immune system called memory reaction cells, continue to produce small quantities of antibodies directed at the pathogen as a preventative measure. Consequently, each individual that is infected with the same pathogen will generate different antibodies directed at different antigens (or epitopes) on the pathogen and thus form unique immune complexes.

It is critical, therefore, for the immune system to recognize which substances are foreign and which are not foreign. That is, the immune system must discriminate between self or self-antigens and non-self or foreign antigens. The failure of the immune system to discriminate between self-antigens and foreign-antigens leads to the production of autoantibodies. These autoantibodies are directed against the body's own reaction cellular components and molecules and are the cause of autoimmune disease.

While studying autoimmune diseases, it was discovered that normal individuals produce autoantibodies that are not associated with disease. Autoantibodies are numerous and specific for self-antigens. The exact role of autoantibodies is unclear. However, because every individual has a unique set of cellular components, each individual has a unique set of autoantibodies directed at those cellular components.

In addition to an animal's large repertoire of antibodies, antibodies directed at foreign-antigens and antibodies directed at self-antigens, it has been discovered that individuals possess "housekeeping" antibodies and "junk" antibodies. These too are unique to each individual. Together, these antibodies and all other antibodies in the body furnish each individual with a unique set of antibodies herein referred to as individual-specific (IS) antibodies.

As described in detail in U.S. Patent No. 5,270,167 to Francoeur, when IS antibodies are allowed to react with a set of random antigens, a certain number of immune complexes form. For example, using a panel of approximately $10^3$ unique antigens, about thirty (30)

immune complexes between IS antibodies in a biological sample that has been diluted 20-fold can be detected. If the biological sample is undiluted, the total number of possible detectable immune complexes that could form would be greater than $10^{23}$. The total number of possible immune complexes can also be increased by selecting "larger" antigens that have multiple epitopes (i.e., a native protein instead of a peptide). Therefore, it will be appreciated that depending on the number and antigens used, the dilution of the biological sample, and the detection method, one skilled in the art can regulate the number of immune complexes that will form and be detected. Collectively the unique immune complexes that form, or fail to form, between IS antibodies and reactants are referred to as an AbP.

5.3.2 *Diagnostic profile*

Like an AbP, a diagnostic profile is generated by an interaction or reaction, or lack of interaction or reaction, between at least one reactant in the reaction vessel and an analyte in the biological sample. What distinguishes an AbP from a diagnostic profile is that an AbP is generated for the purpose of identifying the source of the biological sample, while the diagnostic profile is generated for the purpose of diagnosing the individual or animal. Moreover, the reactants used to generate diagnostic profiles are not limited to antigens.

In one embodiment, diagnostic profiles are generated from immune complexes between antigens in the reaction cell and IS antibodies in the biological sample. In such case, the diagnostic profile is a subset of the AbP. For example, human immunodeficiency virus (HIV). In response to infection by the human immunodeficiency virus (HIV), an individual's immune system will generate antibodies against HIV. As such, those antibodies are part of the individual's unique antibody repertoire, or IS antibodies. Immune complexes that form between antigens in the reaction vessel and HIV specific antibodies in the individual's blood contribute to the individual's unique AbP. However, if the purpose of assaying the blood is to determine whether the individual is infected with HIV, immune complex formation between IS antibodies and HIV specific antigens also generates a diagnostic profile.

Diagnostic profiles may be generated by numerous methods well known in the art, including DNA based assays, drug assays and enzymatic reaction assays which do not involve immune complex formations. Hence analyte for the purposes of the diagnostic profile can be bacterial, viral, or mammalian cell antigens, hormones, drugs, receptors, tumor markers, or

numerous other substances. Moreover, the reactant can be natural or synthetic, a nucleic acid or a peptide, or combinations thereof.

For example, a common method of assaying HIV employs PCR technology. Using this technique, the biological sample is assayed for viral DNA, rather than antibodies directed against the virus. In another example, the biological sample may be tested for therapeutic drugs, endogenous drugs or even illegal contraband. Assays for detecting many drugs, including β-blockers, diuretics, stimulants, narcotic analgesics and anabolic steroids are well known in the art and fall within the scope of the present invention.

### 5.4 Detection and Quantification of Reactant/Analyte Interactions

The prior art is replete with methods for detecting reactions and interactions between two molecules. The present invention may be modified by one skilled in the art to accommodate the various detection methods known in the art. The exact detection method chosen by one in the art will depend on several factors, including the amount of biological sample available, the biological sample type, the stability of the biological sample, the stability of the reactant and the affinity between the reactant and analyte. Moreover, as discussed above, depending on the detection methods chosen, it may be required to modify the reactant and biological sample.

While these techniques are well known in the art, examples of a few of the detection methods which could be utilized to practice the present invention are briefly described below.

#### 5.4.1 *Immunoassays*

There are many types of immunoassays known in the art. The most common type of immunoassay are competitive and non-competitive heterogeneous assays such as enzyme-linked immunosorbent assays (ELISA). In immunoassays the reactant is an antigen. In a noncompetitive ELISA, unlabeled antigen is bound to a solid phase such as a reaction cell in a reaction vessel. Biological sample is combined with antigens bound to the reaction vessel and antibodies (primary antibodies) in the biological sample are allowed to bind to the antigens forming immune complexes. After immune complexes have formed, excess biological sample is removed and the reaction cells are washed to remove nonspecifically bound antibodies. Immune complexes are then reacted with an appropriate enzyme-labeled anti-immunoglobulin (secondary antibody). Anti-immunoglobulins recognize bound antibodies, but not antigens.

Anti-immunoglobulins specific for antibodies of different species, including human, are well known in the art and commercially available from Sigma Chemical Company, St. Louis, MO and Santa Cruz Biotechnology, Santa Cruz, CA. After a second wash step, the enzyme substrate is added. The enzyme linked to the secondary antibody catalyzes a reaction which converts substrate into product. When excess antigen is present, the amount of catalyzed product is directly proportional to the amount of antigen specific antibodies (analyte) in the biological sample. Typically, the reaction product is colored and thus measured spectrophotometrically using UV/VIS technology and equipment well known in the art.

Sandwich or capture assays can also be used to identify and quantify immune complexes. Sandwich assays are a mirror image of non-competitive ELISAs, antibodies are bound to the solid phase and antigen in the blood is measured (analyte). These assays are particularly useful in detecting antigens that are present at low concentrations having multiple epitopes. This technique requires excess antibody to be attached to a solid phase, such as the reaction vessel or magnetic beads. The bound antibody is then incubated with the biological sample and antigens in the biological sample are allowed to form immune complexes with the bound antibody. The immune complex is incubated with an enzyme-linked secondary antibody which recognizes the same or a different epitope on the antigen as the bound antibody. Hence, enzyme activity is directly proportional to the amount of antigen in the biological sample. See Kemeny, DM, and S.J. Challacombe (eds), ELSIA and Other Solid Phase Immunoassays. John Wiley & Sons, Chichester, 1988 which is incorporated by reference.

Typical enzymes that can be linked to secondary antibodies include horseradish peroxidase, glucose oxidase, glucose-6-phosphate dehydrogenase, alkaline phosphatase, β-D-galactosidase and urease. Secondary antigen-specific antibodies linked to various enzymes are commercially available from, for example, Sigma Chemical Company, St. Louis, MO and Amersham Life Sciences, Arlington Height, IL.

Competitive ELISAs are similar to noncompetitive ELISAs except that enzyme-linked antibodies compete with unlabeled antibodies in the blood sample for limited antigen binding sites. Briefly, a limiting number of antigens are bound to the reaction cell. Biological sample and enzyme labeled antibodies are added to the reaction cell. Antigen-specific antibodies in the biological sample compete with enzyme labeled antibodies for the limited number of antigens bound to the reaction cell. After immune complexes have formed, nonspecific

binding is removed, enzyme substrate is added and the enzyme activity is measured. No secondary antibody is required. Because the assay is competitive, enzyme activity is inversely proportional to the amount of antibodies in the biological sample.

Homologous immunoassays can also be used when practicing the method of the present invention. Homogenous immunoassays may be preferred for low molecular weight analytes, such as hormones, therapeutic drugs, and illegal contraband that cannot be analyzed by other methods, or analytes found in high concentration. Homogeneous assays are particularly useful because no separation step is necessary. See Boguslaski, R. C., E.T. Maggio, R. M. Nakamura (eds), Clinical Immunochemistry: Principles of Methods and Applications. Little Brown, Boston (1984) hereby incorporated by reference.

In homologous techniques, bound or unbound antigens are enzyme-linked. When antibodies in the biological sample bind to the enzyme-linked antigen, stearic hinderences inactivate the enzyme. This results in a measurable loss in enzyme activity. Free antigens (i.e., not enzyme-linked) compete with enzyme-linked antigen for limited antibody binding sites. Thus, enzyme activity is directly proportional to the concentration of antigen in the biological sample.

Enzymes useful in homogeneous immunoassays include lysozyme, neuramidase, trypsin, papain, bromelain, glucose-6-phosphate dehydrogenase and β-D-galactosidase. See Persoon, T., Immunochemical Assays in the Clinical Laboratory. Clinical Laboratory Science 5:31 (1992) hereby incorporated by reference. Enzyme-linked antigens are commercially available or can be linked using various chemicals well known in the art including glutaraldehyde and maleimide derivatives.

Fluorescent immunoassays can also be employed when practicing the method of the present invention. Fluorescent immunoassays are similar to ELISAs except that the enzyme is substituted for fluorescent compounds called fluorophores or fluorochromes. These compounds have the ability to absorb energy from incident light and reemit the energy as light of a longer wavelength and lower energy. Fluorescein and rhodamine, usually in the form of isothiocyanates which can be readily coupled to reactants and antibodies are most commonly used in the art. See Stites, D. P. et al., Basic and Clinical Immunology, Appleton & Lange, east Norwalk, CT (1994) hereby incorporated by reference. Fluorescein absorbs light of 490

to 495 nm in wavelength and emits green light at 520 nm in length. Tetramethylrhodamine absorbs light of 550 nm in wavelength and emits red light at 580 nm in length.

Phycobiliproteins isolated from algae, porphyrins, and chlorophyllis which all fluoresce at approximately 600 nm are also being used in the art. See Hemmila, I., Fluoroimmunoassays and Immunofluorometric Assays. *Clin Chem*, 31: 359 (1985) and U.S. Patent No. 4,542,104 to Stryer et al. hereby incorporated by reference. Phycobiliproteins and derivative are commercially available under the names R-phycoerythrin (PE) and Quantum Red™ from for example, Sigma Chemical Company, St. Louis, MO.

In addition, Cy-conjugated secondary antibodies and reactants are useful in immunoassays and are commercially available. Cy-3, for example, is maximally excited at 554 nm and emits light of between 568 and 574 nm. Cy-3 is more hydrophilic than other fluorophores and thus has less of a tendency to bind nonspecifically or aggregate. Cy-conjugated include Cy-2, Cy-3, and Cy-5 are commercially available from Amersham Life Sciences, Arlington Height, IL.

Chemiluminescence, electroluminescence and electrochemiluminescence (ECL) detection methods are also attractive means for quantifying analytes in a biological sample. Luminescence compounds have the ability to absorb energy which is released in the form of visible light upon excitation. In chemiluminescence, the excitation source is a chemical reaction; in electroluminescence the excitation source is an electric field; and in ECL an electric field induces a luminescent chemical reaction.

Molecules used with ECL detection methods generally comprise an organic ligand and a transition metal. The organic ligand forms a chelate with one or more transition metal atoms forming an organometallic complex. Various organometallic and transition metal-organic ligand complexes have been used as ECL labels for detecting and quantifying analytes in biological samples. Due to their thermal, chemical and photochemical stability, their intense emissions and long emission lifetimes, ruthenium, ossium, rhenium, iridium and rhodium transition metals are favored in the art. The types of organic ligands are numerous and include anthracene and polypyridyl molecules and heterocyclic organic compounds. For example, bipyridyl, bipyrazyl, terpyridyl, and phenanthrolyl, and derivatives thereof, are common organic ligands in the art. A common organometallic complex used in the art includes tris-

bipyridineare ruthenium (II), commercially available from IGEN, Inc., Rockville, Md. and Sigma Chemical Company, St. Louis, Mo.

Advantageously, ECL can be performed under aqueous conditions and under physiological pH thus minimizing biological sample handling. See Leland, J. K. et al., "Electrogenerated Chemiluminescence: An Oxidative-Reduction Type ECL Reactions Sequence using Triprophyl Amine," Journal of the Electrochemical Society (1990), vol. 137 #10, pp. 3127-3131, WO 90/05296, and U.S. Patent No. 5541113 to Siddigi et al. hereby incorporated by reference. Moreover, the luminescence of these compounds may be enhanced by the addition of various cofactors such as amines.

In practice, a tris-bipyridineare ruthenium (II) complex, for example, may be attached to a secondary antibody using strategies well known in the art including attachment to lysine amino groups, cysteine sulfhydryl groups, and histidine imidazole groups. In a typical ELISA immunoassay, secondary antibodies would recognize IS antibody bound to antigens but not unbound antigens. After washing nonspecific binding complexes, the tris-bipyridine ruthenium (II) complex would be excited by chemical, photochemical and electrochemical excitation means, such as by applying current to the reaction vessel. *See, e.g.*, WO 86/02734 to Bard, A. J. and Whiteside, G. M., which is herein incorporated by reference. The excitation would result in a double oxidation reaction of the tris-bipyridineare ruthenium (II) complex resulting in luminescence which could be detected by, for example, a photomultiplier tube. Instruments for detecting luminescence are well known and commercially available, for example from IGEN, Inc., Rockville, MD.

### 5.4.2 *Nucleic-based Assays*

In addition to the immunoassays described above, diagnostic profiles may be generated using numerous DNA-based assays, such as polymerase chain reaction techniques. DNA-based assays may be chosen when increased sensitivity is required or when immunoassay detection methods are not suitable. For example, the art has encountered that diagnosing HIV infection in infants is difficult due to the placental passage of IgG antibodies from the infected mother to the child. Infants, therefore, are usually tested for HIV using PCR techniques rather than immunological based assays.

Generally, oligonucleotide primers to conserved regions of HIV genes, such as the *gag* and *pol* genes, are synthesized and used to amplify a region of the gene. The amplified PCR product is then denatured and a radiolabelled DNA probe is added and permitted to hybridize with the amplified product. The hybridized product is identified by running the mixture on a polyacrylamide gel followed by autoradiography. Alternatively, the PCR product can be detected concurrently with amplification using technology known in the art and commercially available from, for example, Idaho Technologies Inc. Wittwer, et al., "Continuous Fluorescence Monitoring of Rapid Cycle DNA Amplification," *BioTechnique*, **22**:130 (1997).

Recent, PCR techniques employing tris-bipyridineare ruthenium (II) complexes have greatly facilitated the procedure and sensitivity of PCR techniques. See Kenten, J. H., et al. "Rapid Electrochemiluminescence Assays of Polymerase Chain Reaction Products", *Clin. Chem.*, **37**: 1626-1632 (1991) which is hereby incorporated by reference. One technique described by T. E. Schutzbank & J. Smith, "Detection of Human Immunodeficiency Virus Type 1 Proviral DNA by PCR Using an Electrochemiluminescence-tagged Probe," *J Clin Microbiol* **33**: 2036-2041 (1995) (which is hereby incorporated by reference) is particularly useful for generating a diagnostic profile. Briefly, oligonucleotide primers directed at conserved regions of an HIV gene are synthesized and used to amplify a region of that gene. One of the oligonucleotides is biotinylated (linked to a biotin molecule) by methods well known in the art. The amplified PCR product is then denatured and hybridized with an ECL-labeled DNA probe which is complementary to the amplified biotinylated DNA stand. After an appropriate hybridization period, the biotinylated-DNA/ ECL-labeled DNA hybrid is reacted with streptavidin coated magnetic particles. A magnetic force is applied to retain the biotinylated-DNA/ ECL-labeled DNA hybrids in the reaction vessel while unhybridized material is removed. Finally, the ECL complexes are excited by chemical, photometric, or electrical means and the photon emission measured.

Similarly, the DNA probe may be labeled with a fluorophore rather than an ECL complex. Custom fluorescein and rhodamine tagged and purified oligonucleotides are commercially available, for example, under the trade name FluoroAmp™ by Promega Corporation, Madison, WI. Other oligonucleotide reporting methods including digoxigenin-11-dUTP, fluorescein-UPT, and oligonucleotide coupled reporter enzymes, such as horseradish peroxidase, are commercially available. Moreover, RNA in the biological sample

may be assayed using reverse transcriptase PCR techniques well known in the art coupled with the techniques described above.

The techniques described above for detecting analytes in a biological sample are only exemplary of the many techniques that could be employed with the present invention. One skilled in the art will appreciate the present invention can be modified to accommodate many other techniques including radioimmune assays (RIA), biotin-antibody conjugated assays, time resolved fluorescence, colloidal gold conjugates assays, ferritin conjugates assays, western blotting, variable number of tandem repeats assays, short tandem repeat assays and sex specific assays using probes for detecting human Y-specific regions.

### 5.5 Digitizing the Results

Once interactions between the reactants and analytes have been identified and quantified, the signals are digitized. The digitized AbP serves as a signature or bar code which identifies the source of the biological sample used to generate the diagnostic profile.

Depending on the reaction vessel used, the digitized data may take numerous forms. For example, the reaction vessel may be a microcassette with 10 columns and 10 rows for a total of 100 reaction cells. Each reaction cell contains at least one reactant. Each reactant is ascribed one specific location which is consistently occupied on the reaction vessel. After biological sample is added to each reaction cell and allowed to incubate, interactions between reactants and analytes in the biological sample are identified and quantified. In each reaction cell, an interaction between the reactant(s) in that cell and analytes in the biological sample either did or did not yield a quantifiable signal. As illustrated in Figure 5, in one preferred embodiment, the results of the AbP and diagnostic profile are digitized by ascribing each one of the 100 reaction cells a numerical value of either "0" if a quantifiable signal was not obtained or "1" if a quantifiable signal was obtained. Using this method, therefore, the digitized AbP and diagnostic profile of a human or other animal will comprise a unique set of "0" and "1."

The numerical values "0" or "1" will, of course, preferably be normalized to signals obtained in internal control reaction cells so that digitized AbPs obtained at a later time can be properly compared. For example, one or several of the reaction cells will contain a known antigen which will remain constant over time. Therefore, if a subsequent biological sample is

mor or less dilute than a previous biological sample, the signals can be normalized using the signals from the known antigen.

It will be appreciated by one skilled in the art that other methods of digitizing the AbP and diagnostic profile exist and may be employed. For example, rather than ascribing each cell a numerical value of "0" or "1," the numerical value may be incremental and directly proportional to the strength of the signal.

By digitizing the AbP signals, the biochemical results can be entered into a computer and quickly accessed and reference. Within seconds of having the AbP digitized, a computer can compare a previously digitized AbP to ensure the biological sample was indeed obtained from the intended source and not misidentified during handling. Indeed, by searching the entire digitized databank, it would even be possible to positively identify the source of the biological sample. Thus, the method of the present invention can both discriminate and positively identify the source of a biological sample.

## 6. SUMMARY

In summary, misidentification of biological samples is a serious problem in the art which results in thousands of misdiagnosis a year. Hence, there is great need in the art for a method of identifying the source of the biological sample. The present invention provides such a method by linking diagnostic results to a biological signature. The invention is based on the principle that humans and other animals have a unique set of antibodies referred to as individual-specific antibodies or IS antibodies. When IS antibodies are reacted with a random number of antigens, certain IS antibodies specifically bind to certain antigens forming immune complexes. The highly unique combination of immune complexes that form, or fail to form, generate a unique biological signature called an antibody profile (AbP) or antibody fingerprint.

The present invention identifies the source of the biological sample by generating an AbP of the biological sample and linking the AbP to the diagnostic results. Therefore, subsequent AbP linked diagnostic test results can be compared to previous or subsequent AbPs to ensure the biological sample tested was obtained from the same source or the source in question. If the AbPs do not match, the biological samples were mislabeled in transit or derived from a different source. Accordingly, the source of the biological sample can be traced using the AbP or another biological sample can be obtained and tested.

In addition to absolute concordance between biological samples and diagnostic testing, the present invention is fast, cost effective, requires small volumes of biological samples, requires little training to perform, and can be easily automated. By directly identifying the source concurrent to the diagnostic assay as well as immediate reporting, the method of the present invention addresses the largest source of laboratory errors, including pre- and post-analytical errors.

What is claimed is:

1. A method for assaying analytes in a biological sample and simultaneously identifying the origin of the biological sample assayed comprising:

a. obtaining a plurality of reactants;

b. adding the reactants to a reaction vessel having a plurality of reaction cells, a first subset of reaction cells containing at least one reactant for the purpose of obtaining an antibody profile and a second subset of reaction cells containing at least one reactant for the purpose of obtaining a diagnostic profile;

c. contacting each reaction cell with the biological sample for a time sufficient to allow analytes in the biological sample to react with reactants in the reaction cells;

d. generating an antibody profile and a diagnostic profile by detecting whether analytes in the biological sample reacted with reactants in the reaction cells.

2. The method for assaying analytes in a biological sample of claim 1 comprising digitizing the resulting antibody profile and the diagnostic profile such that the digitized antibody profile can be compared with other digitized antibody profiles for the purpose of identifying the source of the biological sample.

3. The method for assaying analytes in a biological sample of claim 1 wherein the reactant added to the first subset of reaction cells is an antigen.

4. The method for assaying analytes in a biological sample of claim 1 wherein the reactant is bound to the reaction vessel.

5. The method for assaying analytes in a biological sample of claim 1 wherein the analytes are individual specific antibodies.

6. The method for assaying analytes in a biological sample of claim 1 wherein the biological sample is derived from the group consisting of blood, tissue, saliva, semen, serum, plasma, urine, amniotic, pleural and cerebrospinal fluid.

7. The method for assaying analytes in a biological sample of claim 1 wherein the reactants are synthesized directly in the reaction cells.

8. The method for assaying analytes in a biological sample of claim 1 wherein the antibody profile and the diagnostic profile are detected by different methods.

9. The method for assaying analytes in a biological sample of claim 7 wherein the antibody profile is detected by an immunoassay.

10. The method for assaying analytes in a biological sample of claim 7 wherein the diagnostic profile is detected by a nucleic acid-based assay.

11. A method for assaying analytes in a biological sample and simultaneously identifying the origin of the biological sample assayed comprising:

a. obtaining a plurality of antigens;

b. obtaining a plurality of reactants;

c. adding the antigens and the reactants to a reaction vessels having a plurality of reaction cells, a first subset of reaction cells containing at least one antigen for the purpose of obtaining an antibody profile and a second subset of reaction cells containing at least one reactant for the purpose of obtaining a diagnostic profile;

d. contacting the first subset of reaction cells with the biological sample for a time sufficient to allow individual specific antibodies in the biological sample to form immune complexes with antigens in the reaction cells;

e. generating an antibody profile by detecting whether individual specific antibodies in the biological sample formed immune complexes with the antigen in the reaction cells;

f. contacting the second subset of reaction cells with the biological sample for a time sufficient to allow analytes in the biological sample to react with the reactants in the reaction cells;

g. generating a diagnostic profile by detecting whether analytes in the biological sample reacted with reactants in the reaction cells.

12. The method for assaying analytes in a biological sample of claim 11 comprising digitizing the resulting antibody profile and the diagnostic profile such that the digitized antibody profile can be compared with other digitized antibody profiles for the purpose of identifying the source of the biological sample.

13. The method for assaying analytes in a biological sample of claim 11 wherein the reactants are bound to the reaction vessel.

14. The method for assaying analytes in a biological sample of claim 11 wherein the biological sample is derived from the group consisting of tissue, blood, saliva, semen, serum, plasma, urine, amniotic, pleural and cerebrospinal fluid.

15. The method for assaying analytes in a biological sample of claim 11 wherein the reactants are synthesized directly in the reaction cells.

16. The method for assaying analytes in a biological sample of claim 11 wherein the diagnostic profile is detected by a nucleic acid-based assay.

17. The method for assaying analytes in a biological sample of claim 11 wherein the nucleic acid-based assay is a polymerase chain reaction assay.

18. A method for assaying analytes in a biological sample and simultaneously identifying the origin of the biological sample assayed comprising:

a. obtaining a plurality of antigens;

b. obtaining oligonucleotides directed at the analyte in the biological sample to be assayed;

c. adding the antigens and oligonucleotides to a reaction vessel having a plurality of reaction cells, a first subset reaction cells containing at least one antigen for the purpose of obtaining an antibody profile and a second subset of reaction cells containing oligonucleotides for the purpose of obtaining a diagnostic profile;

d. contacting the first subset of reaction cells with the biological sample for a time sufficient to allow individual specific antibodies in the biological sample to form immune complexes with antigens in the reaction cells;

e. generating an AbP by detecting whether individual specific antibodies in the biological sample formed immune complexes with the antigen in the reaction cell;

f. contacting the second subset of reaction cells with the biological sample and attempting to amplify the analyte using the oligonucleotides in the reaction cells and polymerase chain reaction techniques;

g. generating a diagnostic profile by detecting whether analytes in the biological sample was amplified.

19. The method for assaying analytes in a biological sample of claim 18 comprising digitizing the resulting antibody profile and the diagnostic profile such that the digitized antibody profile can be compared with other digitized antibody profiles for the purpose of identifying the source of the biological sample.

20. The method for assaying analytes in a biological sample of claim 18 wherein the biological sample is derived from the group consisting of tissue, blood, saliva, semen, serum, plasma, urine, amniotic, pleural and cerebrospinal fluid.

21. The method for assaying analytes in a biological sample of claim 18 wherein the reactant is bound to the reaction vessel.

22. The method for assaying analytes in a biological sample of claim 18 wherein the analytes are individual specific antibodies.

23. The method for assaying analytes in a biological sample of claim 18 wherein the one of the oligonucleotides is coupled to biotin, digoxigenin-11-dUTP, fluorescein-UPT, a reporter enzyme.

24. The method for assaying analytes in a biological sample of claim 20 wherein the diagnostic profile detection method further comprising hybridizing an electrochemiluminescent-labeled DNA probe which is complementary to the amplified analyte.

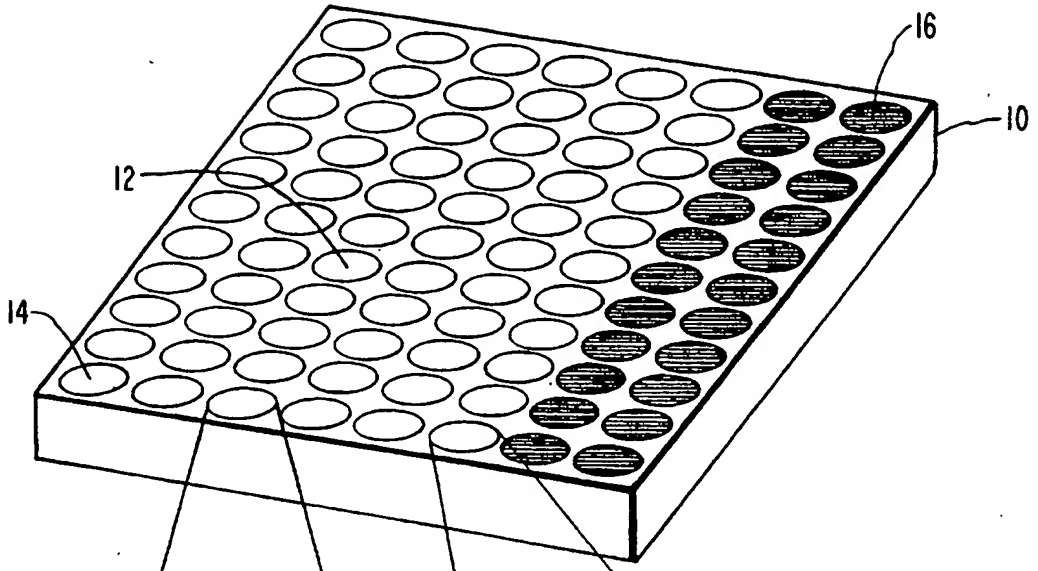



FIG. 1

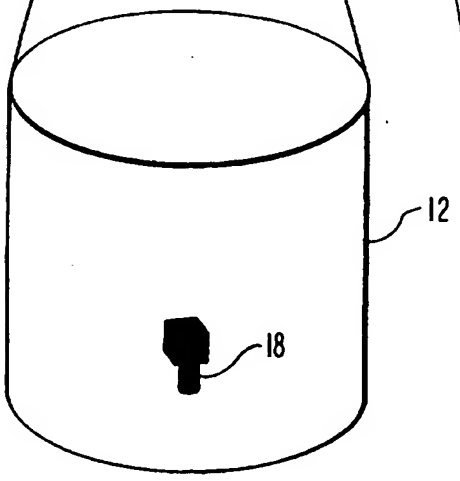



FIG. 1A

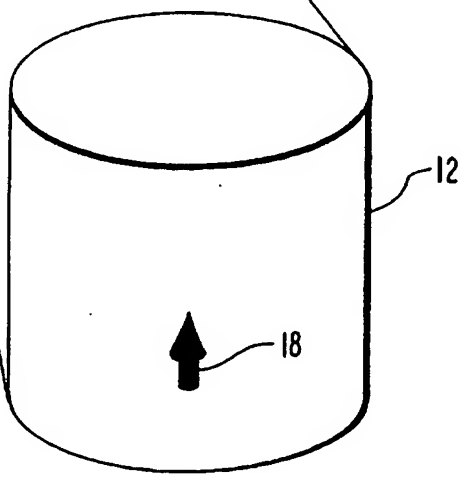
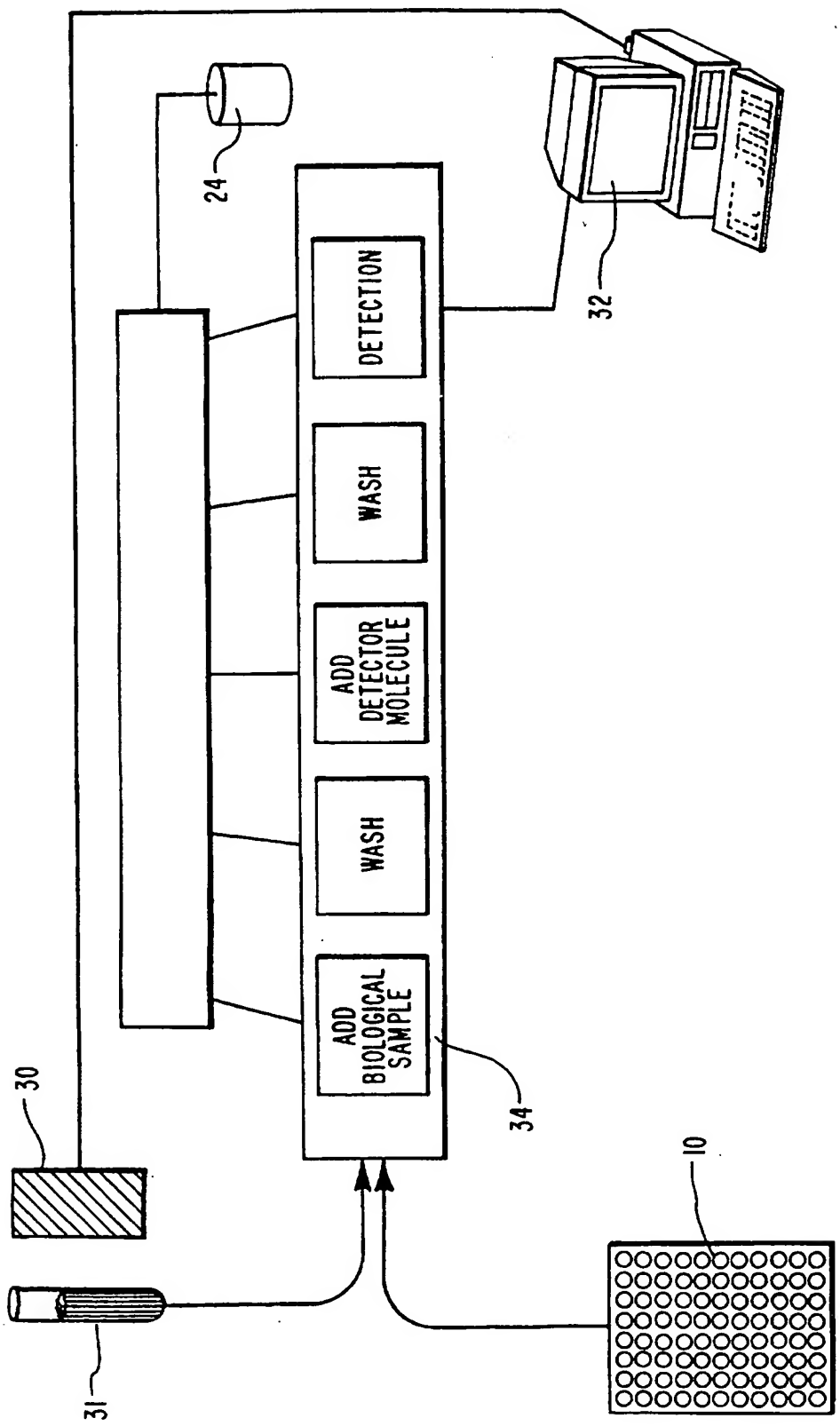



FIG. 1B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ADD BIOLOGICAL SAMPLE
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30
31
10

FIG. 2

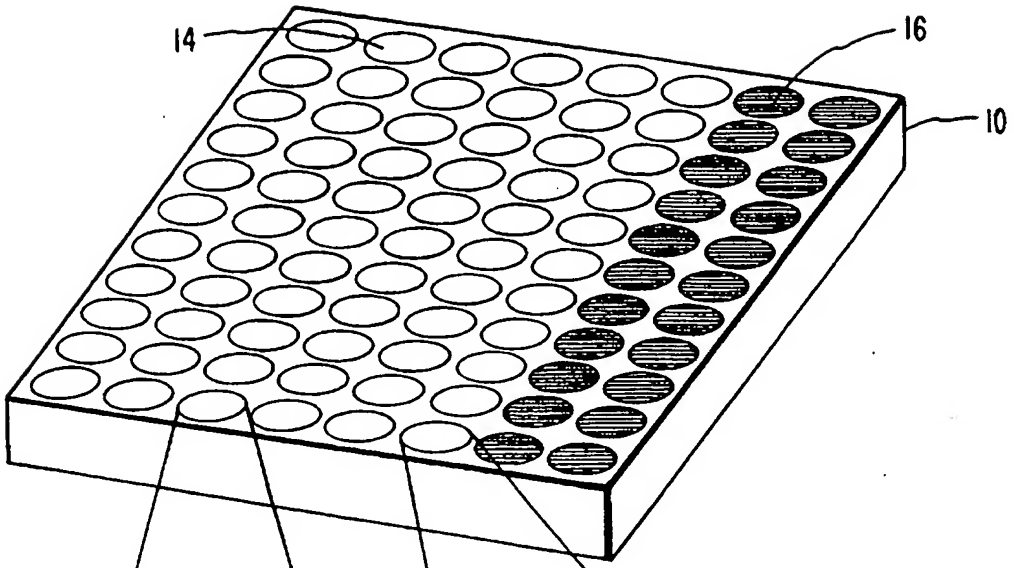



FIG. 3

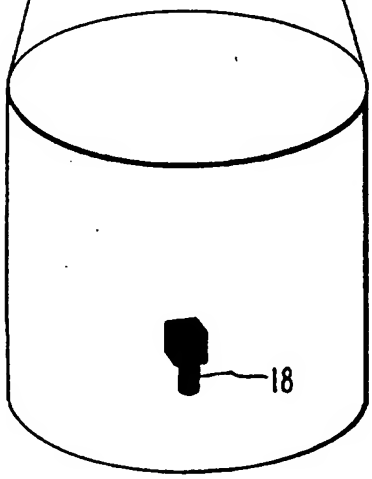



FIG. 3A

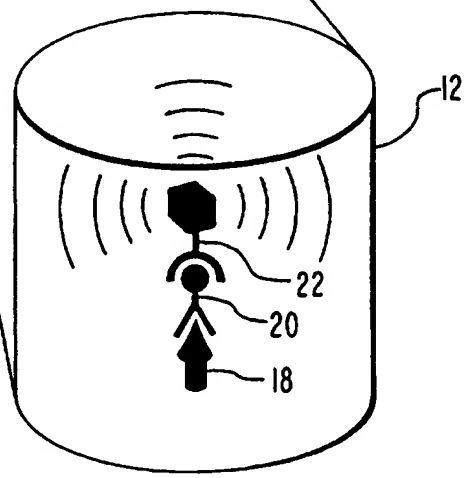
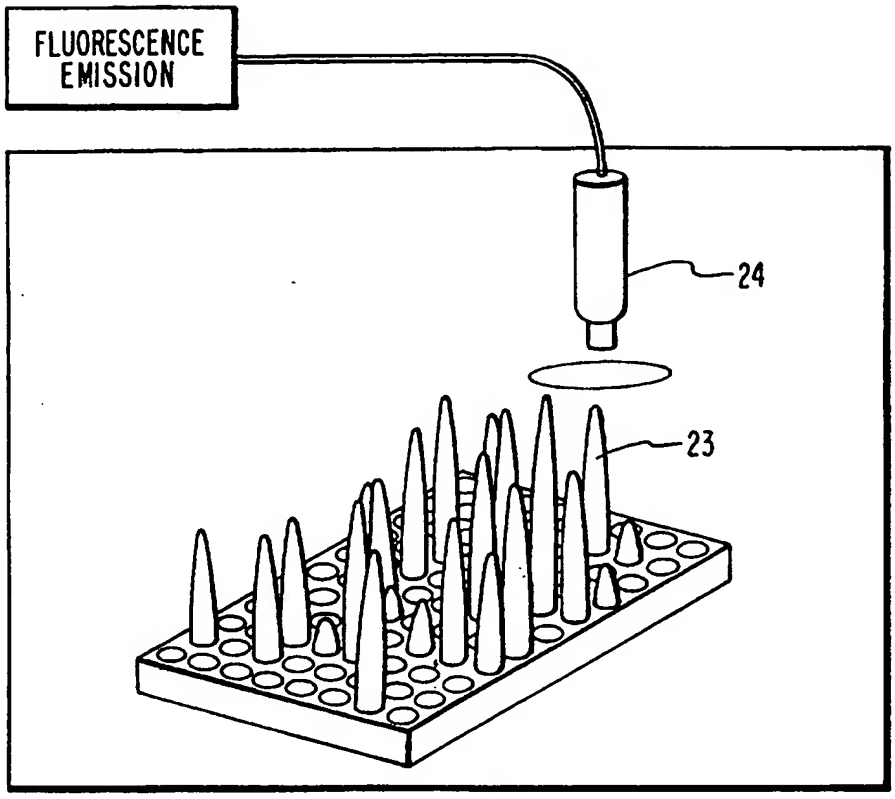



FIG. 3B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23

FIG. 4

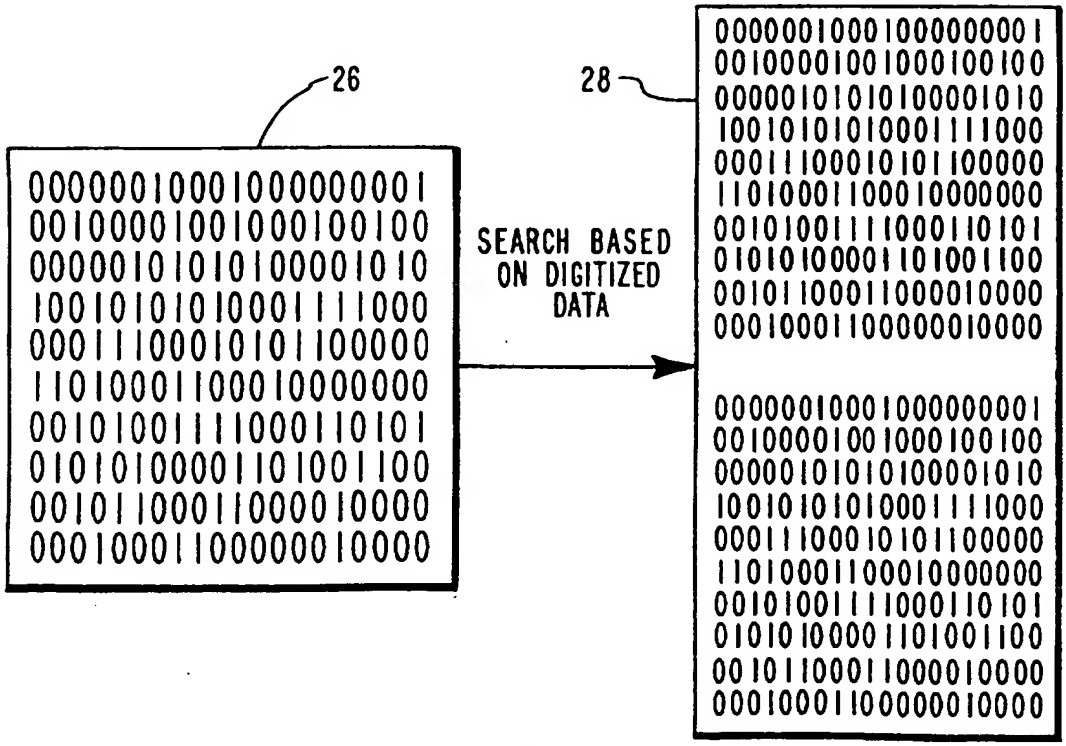
26
28
SEARCH BASED ON DIGITIZED DATA

FIG. 5

# INTERNATIONAL SEARCH REPORT

International application No.
PCT/US97/02138

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) :Please See Extra Sheet.
US CL :435/4, 6, 7.1, 7.2, 7.32, 7.92, 89, 91.1, 91.2

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 435/4, 6, 7.1, 7.2, 7.32, 7.92, 89, 91.1, 91.2

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

DIALOG, STN, CAPLUS, MEDLINE, EMBASE, BIOSIS, SCISEARCH, WPIDS
Search Terms: Antibody Profile, Antibody Fingerprint, PCR, Polymerase Chain Reaction

C. DOCUMENTS CONSIDERED TO BE RELEVANT

| Category* | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|---|---|---|
| X | BISHOP ET AL. Detection of a carrier state in Theileria parva-infected cattle by the polymerase chain reaction. Parasitology. 1992. Vol. 104. pages 215-232. See entire document. | 1-24 |
| X | TOBE ET AL. Single-well genotyping of diallelic sequence variations by a two-color ELISA-based oligonucleotide ligation assay. Nucleic Acids Research. 1996. Vol. 24 No. 19. pages 3728-3732. See entire document. | 1-24 |
| X | NIKIFOROV ET AL. Genetic Bit Analysis: A solid phase method for typing single nucleotide polymorphisms. Nucleic Acids Research. 1994. Vol. 22 No. 20. pages 4167-4175. See entire document. | 1-24 |

[X] Further documents are listed in the continuation of Box C. [ ] See patent family annex.

| | | | |
|---|---|---|---|
| * | Special categories of cited documents: | "T" | later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention |
| "A" | document defining the general state of the art which is not considered to be of particular relevance | "X" | document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone |
| "E" | earlier document published on or after the international filing date | "Y" | document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art |
| "L" | document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified) | "&" | document member of the same patent family |
| "O" | document referring to an oral disclosure, use, exhibition or other means | | |
| "P" | document published prior to the international filing date but later than the priority date claimed | | |

Date of the actual completion of the international search
14 APRIL 1997

Date of mailing of the international search report
05 MAY 1997

Name and mailing address of the ISA/US
Commissioner of Patents and Trademarks
Box PCT
Washington, D.C. 20231
Facsimile No. (703) 305-3230

Authorized officer
VERLENE RYAN
Telephone No. (703) 308-0196

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INTERNATIONAL SEARCH REPORT

International application No.
PCT/US97/02138

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

| Category* | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|---|---|---|
| X | VAN DOORN ET AL. Analysis of Hepatitis C virus genotypes by a line probe assay and correlation with antibody profiles. Journal of Hepatology. 1994. Vol. 21. pages 122-129. See entire document. | 1-24 |

# INTERNATIONAL SEARCH REPORT

International application No.
PCT/US97/02138

A. CLASSIFICATION OF SUBJECT MATTER:
IPC (6):

C12P 19/30, 19/34; C12Q 1/00, 1/68; G01N 33/53, 33/554, 33/569, 33/537, 33/543